Supplement to the Annals of the Transvaal Museum.
Proceedings of the Transvaal Biological Society.

ANNALS
OF
The Transvaal Museum.

VOL. II. FEBRUARY, 1910. No. 2.

ON THE DEVELOPMENT OF PIROPLASMA IN THE DIFFERENT ORGANS.

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SINCE the investigations of R. Koch on East Coast fever of cattle in East Africa, the cause of this disease has formed the subject of much discussion.

At present there exist two different views. Some investigators hold that East Coast fever is due to an invisible organism, others declare *P. parvum* to be the cause, but neither party has been able to prove to the satisfaction of the other that their investigations were free of errors. This is due to the fact that the cattle of Africa are, as a rule, infected with various parasites; with *P. bigeminum*, and the more or less harmless parasite *P. mutans*, which, morphologically, is very difficult to distinguish from *P. parvum*. Moreover, there is much controversy on the so-called plasma granules or Koch's bodies, also known as blue bodies, which are found in the organs of cattle suffering from East Coast fever.

In this short publication, which will be succeeded by a more detailed one, I wish to communicate some investigations on the blue bodies of Koch which will throw some light on their nature. For the present I shall leave out details, neither can I enter into the literature on the subject, which will have to be considered in the more exhaustive report.

Experiments were carried out since March of this year in the Transvaal Veterinary Bacteriological Laboratory. The material used consisted in cattle whose origin we know, and in ticks whose history we were fully acquainted with; accordingly, double infections could be excluded from the very start. The ticks placed on control cattle directly imported from England proved that they were exclusively infected with East Coast fever. In addition to this, Dr. Theiler gave me permission to utilise the cattle used in various experiments on East Coast fever which had been infected experimentally through the implantation and inoculation of different organs. For my zoological studies, therefore, a considerable amount of material was available.

Before discussing the question of Koch's bodies, I wish to make some remarks on *P. parvum*. Last year Ollwig, at the Congress of Microbiologists, in Berlin, stated that *P. parvum* and *P. mutans* were identical. My experiments leave no doubt that *P. parvum* and *mutans* are two different organisms, which view has been held for a number of years by different authors. Blood containing *P. parvum* in extraordinarily large numbers was injected subcutaneously and into the organs of various animals, and in no instance was an infection transmitted. On the contrary, it was an easy matter to transmit *P. mutans* to a healthy beast. An emulsion of 1 c.c. red corpuscles in 10 c.c. saline solution was sufficient to do so. As a further proof, it may be stated that *Rhipicephalus appendiculatus* in the Transvaal transmit *P. mutans* only in exceptional cases.

P. parvum and P. mutans are, therefore, with regard to their pathogenic effect, two different parasites, which also show differences in their cycle of development.

Together with the daily blood examinations, systematic punctures of glands and spleen were made. On comparing preparations from cattle suffering from East Coast fever with those infected with *P. mutans*, no doubts can be left about the cause of East Coast fever. Shortly before the parasites appear in the blood, one will notice segmenting forms of Koch's bodies which throw off a great number of parasites. For this investigation the large shoulder glands are the most suitable. Neither an infection with *P. bigeminum* nor one with *P. mutans*, *P. equi*, or *P. canis*, shows similar evolution forms, whereby it is proved that Koch's bodies represent a stage in the development of *P. parvum*, as already Koch himself had surmized. Accordingly, the view of Martin Mayer that the so-called Koch's granules are not specific for East Coast fever falls to the ground.

The blue bodies represent indeed a certain stage in the cycle of P. parvum which corresponds to the shizogony in the cycle of the malarial and other parasites.

These bodies (fig. 1–2 and fig. 5 *c–d*) appear both intracellularly and extracellularly, more particularly in the lymphocytes. Quite exceptionally polynuclear leucocytes are found to be infected. The development takes place more or less in the following manner. In the blue body (Agamont) a nucleus is first formed ; this grows and becomes richer in chromatin and plasma ; subsequently it divides successively into a great number of smaller nuclei similarly to what occurs in the cycle of haemoproteus which takes place in the organs of the pigeon and padda. The cells thus formed break up into as many sub-divisions as there are nuclei (fig. 4–5*a*). One encounters and but rarely a second one to which I will refer hereafter. The explanation of this fact may be that this shizogonous stage, which I call, with Hartmann, agamogony, repeats itself. Accordingly agametes, resulting from the agamogonous stage, would grow into agamonts. I have, however, not been able to completely follow up the further development of these latter forms.

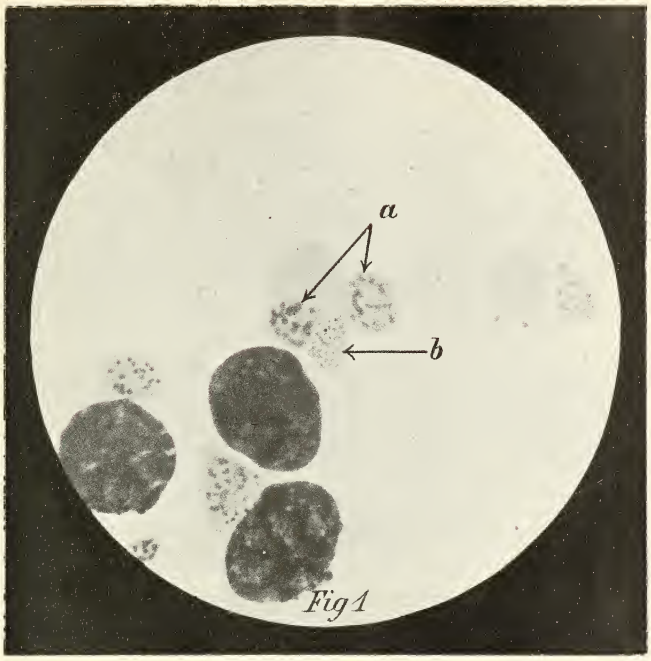
The second mode of multiplication ends with the segmentation into a great number of small parasites undoubtedly resulting from the agamogonous multiplication (fig. 3–5). The young parasites enter into the red corpuscles and represent now the forms known as *P. parvum*. Thus we have in the organs, particularly in the lymphatic glands and the spleen, two different phases, which morphologically are not difficult to distinguish from each other. The nuclei of the one phase (agamogonous) when young,

show a loose structure and are irregularly shaped. The nuclei of the second phase, that is of the second generation or gamagonous, take the nuclear stains more deeply; they undergo reductions and accordingly appear smaller. Before the segmentation of the nuclei has taken place, they are oval-shaped. One is struck by the fact that the extracellular segmenting forms of the gamogonous generation produce parasites in far smaller numbers (fig. 3) than those found in the cells themselves (fig. 4—5*b*). I have not been able to give an explanation of this observation. In the blood no multiplication has as yet been seen, but my studies on these parasites are not yet concluded. Plasma granules were found in the blood, but this fact by no means necessitates a change of the above technology. It may also be possible that two agamogonous stages have to be distinguished, and the parasites which develop in the red corpuscles would have to be considered as the gamogonous generation. I am, however, of the opinion that the two stages in the cycle which I have called provisionally agamogony and gamogony, and which finally lead to the formation of the forms seen in the blood, succeed each other within the cell. Accordingly it is clear that the number of parasites increase as the disease develops. The forms which result from the segmentation of the last generation differ morphologically in no way from the parasites seen in the blood at the commencement of an infection. They possess, in proportion to the whole parasite, a large nucleus which contains a distinctive karyosome. In using Giemsa's stain one can see differences due to the staining in the plasma of the bodies belonging to the agamogonous stage as well as of the gamogonous one. There are dark blue forms along with light blue ones containing larger alveli. It is possible that from the very start we have to deal with a sexual differentiation. The above nuclear phenomen can easily be studied in preparations stained according to the dry and moist methods of Giemsa, as well as in those stained with haemotoxylin. Microscopic examination of the various organs, particularly of the spleen, does not allow of definite conclusions. These organs represent a filter which retains the broken down parasites usually encountered in all protozoic diseases. When using the vital method I was never able to recognize the blue bodies, so that it is impossible for me to believe that these represent an assimulation product of the nuclei. The presence of Koch's bodies in the kidneys is of secondary importance. In East Coast Fever experimentally produced, either through the implantation of organs or in cases brought on by ticks, which finish rapidly with death, the formation of infarcts in the kidneys may not take place at all. My drawings of, and the communication referring to Koch's granules, concern fresh material obtained with a syringe from the lymphatic glands and the spleen. In cases of East Coast fever, where fever is present without parasites being present in the blood, the diagnosis can be made by examining juice from the spleen and lymphatic glands. If in the juice obtained from the puncture of the lymphatic glands or the spleen are seen Koch's bodies containing only larger nuclei of a loose structure, and no forms of the second or gamogonous generation, it is possible that this latter does not take place at all, and no parasites are then found in the blood. Whether ticks which have been sucking the blood of such animals transmit the disease will be shown by later experiments.

It remains to explain the fact that the blood of animals suffering from East Coast fever injected into healthy animals does not produce the disease. It is possible that the blood contains forms which can only develop in the tick, and which, injected into the animal, die. In the

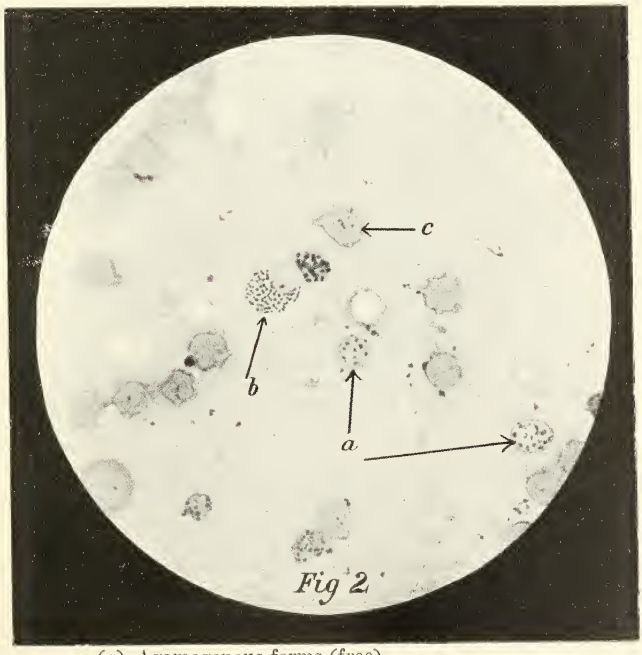
transplantation of organs undertaken by Theiler and Meyer, Koch's granules are directly transmitted. These represent, under natural conditions, the stages which are formed only after the tick has transmitted the disease. The infection of the various glands in the body of an animal takes place through the blood current and accordinglyit becomes feasible to expect that the inoculation of blood also ought toproduce the disease. A suitable occasion would probably arise when the plasma granules are found in the blood. Experiments to elucidate this will be carried out as soon as opportunity occurs. In an animal which was infected by the transplantation of organs I found Koch's granules in the blood. Dr. Meyer showed me blood smears sent in from the practice in which a great number of mononuclear leucocytes contained the plasma bodies.

Concerning the place of the East Coast fever parasite in protozology, the proposition of Bettencourt and others to separate it from the piroplasms and to substitute a new name (*Theileria parva*) is justified.

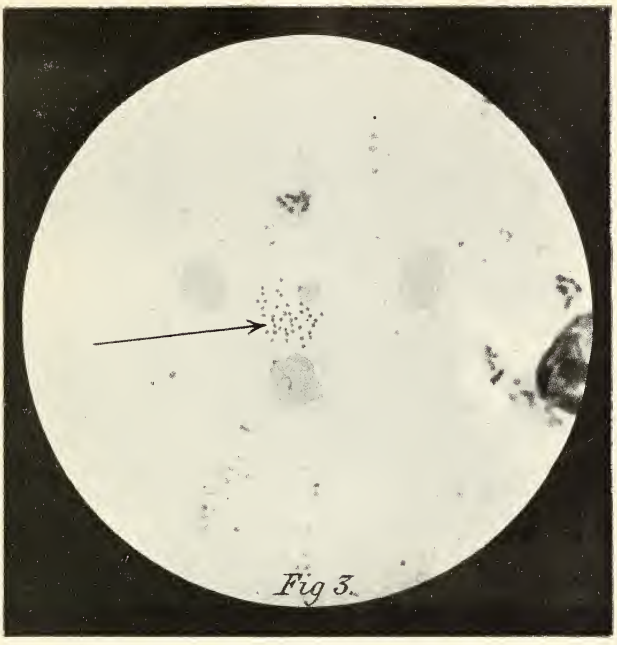


Koch's Bodies (free).

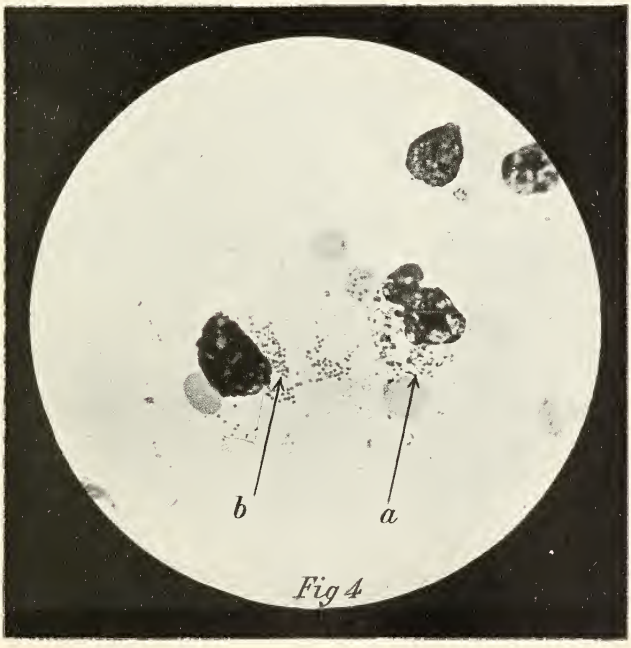
(*a*) Agamogonous forms.
(*b*) Gamogonous form.



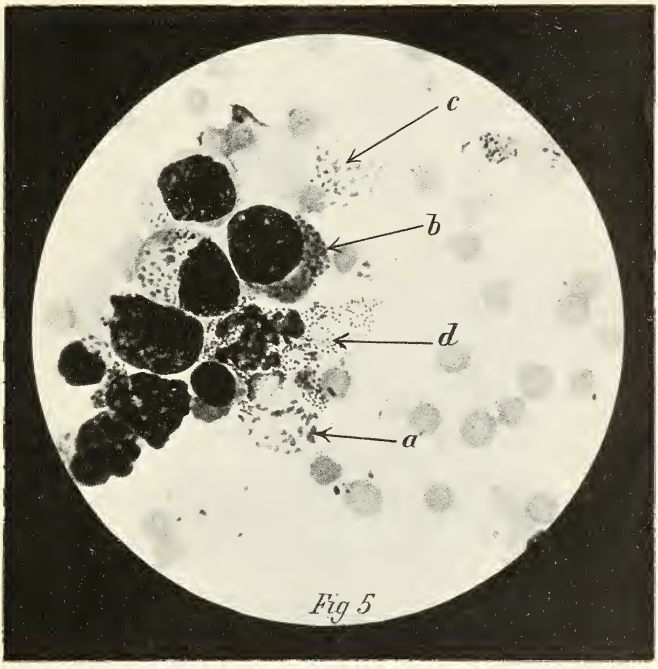
(*a*) Agamogonous forms (free).
(*b*) Gamogonous form before the segmentation.
(*c*) Blood corpuscle with parasites.



Free gamogonous form in segmentation.



(*a*) Intracellular agamogonous form in segmentation.
(*b*) Intracellular gamogonous form in segmentation.



(*a*) Intracellular agamogonous form.
(*b*) Intracellular gamogonous form.
(*c*) Free agamogonous form in segmentation.
(*d*) Intracellular form in segmentation.